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Genetics

Construction of a clonable, infectious, and tumorigenic mouse mammary tumor virus provirus and a derivative genetic vector

(glucocorticoid induction/helper virus/retrovirus/mammary neoplasms)

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ABSTRACT **Molecular genetic studies of mouse mammary tumor virus (MMTV) have been hampered by the difficulty of cloning proviruses of milk-borne strains because of inhibitory sequences located in the *gag* gene. To surmount this barrier we have constructed a hybrid MMTV provirus composed of clonable 5′ sequences (encompassing *gag*) from an endogenous MMTV provirus of C3H mice (*Mtv-1*) and 3′ sequences from the milk-borne strain of MMTV in C3H mice, MMTV(C3H). Virions produced from XC cells transfected with this hybrid provirus are infectious in cell culture and tumorigenic in BALB/cJ mice. A vector derived from this provirus, containing the neomycin phosphotransferase gene (*neo*) and origins of replication from simian virus 40 and pBR322, is capable of transferring G418 resistance by virus infection in cell culture when supplied with viral proteins from either MMTV(C3H) or the hybrid MMTV. Expression of both hybrid and vector proviruses is inducible by dexamethasone in infected cells.**

Genetic manipulation of the mouse mammary tumor virus (MMTV) genome would be useful in determining the roles of MMTV nucleic acid sequences and gene products in the viral life cycle, tissue tropism, and pathogenesis. Such work has been largely precluded, however, by the failure to obtain complete molecular clones of fully competent MMTV proviruses. This is due to a region in the *gag* gene of some strains of MMTV that is extremely resistant to propagation in cloning vectors (1–4). The molecular basis for this resistance is unknown, but perturbation of these sequences by deletion, insertion of bacterial insertion sequences, base substitutions, or other rearrangements can allow the DNA to be cloned (ref. 5 and references therein). Unfortunately, such changes usually render the provirus defective for replication.

To create a clonable MMTV provirus that might produce virus with complete biological activity, we have constructed a hybrid MMTV provirus composed of sequences from the endogenous provirus *Mtv-1* and from milk-borne MMTV(C3H). We show here that the hybrid MMTV produced from this construct is infectious and capable of inducing mammary tumors in mice with latency similar to that of MMTV(C3H). We also have constructed an MMTV shuttle vector that contains the neomycin phosphotransferase gene (*neo*) and origins of replication from simian virus 40 (SV40) and pBR322. The MMTV-neo vector RNA can be packaged with proteins supplied by MMTV(C3H) or hybrid MMTV, and these particles can subsequently infect susceptible cells. Expression of both vector and helper proviruses, like wild-type MMTV DNA, is regulated by glucocorticoid hormones.

MATERIALS AND METHODS

Plasmids. p15R contains the right half of an integrated MMTV(C3H) provirus (from the single *Eco*RI site in *pol*) and flanking rat sequences isolated from an infected XC cell line (ref. 3; gift of J. Majors, Washington University, Saint Louis). pL635-9-3 contains the left half of the endogenous provirus *Mtv-1* (to the *Eco*RI site) and 0.3 kilobase (kb) of flanking sequences isolated from a C3H mouse (ref. 1; gift of G. Hager, National Cancer Institute). pZIPneoSV(X) is a Moloney murine leukemia virus (Mo-MLV)-based shuttle vector (ref. 6; gift of C. Cepko, Harvard Medical School). pUCHSG (gift of E. Liu, University of North Carolina, Chapel Hill) contains the hygromycin B phosphotransferase gene controlled by a thymidine kinase promoter in pUC18 and is derived from pHyg (7).

Hybrid MMTV Provirus and MMTV-neo Vector Construction. The hybrid MMTV and MMTV-neo vector proviruses were constructed by using appropriate portions of the plasmids described above. Details of the constructions, beyond those provided in the text and legend to Fig. 1, will be provided to interested readers upon request.

Cell Culture, Transfections, and Infections. XC cells were derived from a rat sarcoma induced by Rous sarcoma virus (8). D108 cells are a clone of MMTV(C3H)-infected XC cells producing MMTV (9). All cells were grown in Dulbecco's modified Eagle's medium with 10% (vol/vol) fetal bovine serum, penicillin, and streptomycin. Cells containing MMTV proviruses were also supplied with 0.1 μM dexamethasone.

DNAs were introduced into XC cells by calcium phosphate transfection (10). Selections were performed in the presence of G418 (Geneticin; GIBCO) at 300 μg/ml (net concentration) and/or hygromycin B (Calbiochem) at 400 μg/ml. G418-resistant colonies of cells transfected with the MMTV-neo vector provirus (and the hybrid MMTV provirus, if appropriate) were cloned and screened for production of MMTV-neo vector and hybrid MMTV virions into the medium. Virions in 24-hr supernatants from confluent cultures were pelleted by centrifugation at 200,000 × *g*, extracted with phenol/chloroform, 1:1 (vol/vol), and ether, applied to a nylon membrane, and hybridized as described below for DNA analysis by using a probe for the *neo* or *env* gene. Typical physical virus titers from producer cell lines, when compared with DNA standards by hybridization analysis, were $\approx 5 \times 10^6$ particles per ml for the hybrid MMTV and 10^4 particles per ml for MMTV-neo. Biological titers were not determined.

XC cells were infected by cocultivation. Recipient XC cells were first made resistant to hygromycin B by transfection with the plasmid pUCHSG. Donor XC cells, containing MMTV-neo vector provirus and either hybrid MMTV provirus (introduced by transfection) or MMTV(C3H) (previously introduced by infection; cell line D108), are resistant to G418 because of the *neo* gene in the MMTV-neo vector. Donor and recipient cells were cocultivated at a 1:1 ratio in nonselective medium until confluent. Cells were then pas-

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Abbreviations: MMTV, mouse mammary tumor virus; Mo-MLV, Moloney murine leukemia virus; LTR, long terminal repeat; SV40, simian virus 40.

saged 1:10, grown to confluence, and passaged 1:20 into medium containing hygromycin B (400 μg/ml) and G418 (300 μg/ml, net concentration). Hygromycin B kills all donor cells, and G418 kills all recipient cells except those that have acquired an MMTV-neo vector provirus. Occasionally, doubly resistant cells arise by cell fusion but are easily identified by DNA blot analysis.

Animal Infection. Three to 4-week-old BALB/cJ female mice (The Jackson Laboratory) were infected with the hybrid MMTV by subcutaneous and intraperitoneal injection of rat XC cells producing the virus; 2–4 × 10^6 cells, suspended in 0.1 ml of Dulbecco's phosphate-buffered saline, were injected into each of five sites per mouse: four subcutaneous injections near the mammary glands proximal to each leg and one intraperitoneal injection. The minimal infective dose and the most efficient site of infection were not determined.

DNA Isolation, Analysis, and Hybridization Probes. High molecular weight DNAs from cultured cells, tissues, and tumors were isolated by standard procedures (11). Samples (10 μg) digested with restriction endonucleases were separated by electrophoresis in 0.8% agarose gels, blotted onto nylon membranes (GeneScreen; New England Nuclear), and crosslinked to the membrane with ultraviolet light (12). Probe labeling and hybridization conditions were as described (13) except that the hybridization buffer contained 0.5 M sodium phosphate (pH 7.2), 1 mM EDTA, 1% bovine serum albumin, 7% $NaDodSO_4$, and 10% formamide.

The *neo* hybridization probe was isolated from the MMTV-neo vector construct as a 1.7-kb *Xho* I–*Eco*RI fragment. The *env* probe was a 1.9-kb *Pst* I fragment from the hybrid provirus. A 1.4-kb *Pst* I fragment from the hybrid provirus was used as a probe for long terminal repeats (LTRs). The *gag-pol* probe was a 3.1-kb *Acc* I fragment from the hybrid provirus. All fragments were gel-purified before labeling.

RESULTS

Strategy for Hybrid MMTV Provirus and MMTV-neo Vector Construction. To circumvent the problem of the inhibitory sequence present in the *gag* region of most MMTV proviruses, we constructed a hybrid MMTV provirus in which this sequence is replaced by the *gag* region from *Mtv-1*, an endogenous provirus that appears to present no difficulties during molecular cloning. The hybrid MMTV construct contains the *Mtv-1* sequence upstream of the *Eco*RI site in *pol* and sequences from the milk-borne virus from C3H mice [MMTV(C3H)] downstream from this site (Fig. 1). After one round of viral replication, the only *Mtv-1* sequences remaining in the newly generated provirus will be those of *U5* and other 5′ nontranslated regions, *gag*, and a portion of *pol* up to the *Eco*RI site. The *U3* region, containing transcriptional control sequences, will be derived from MMTV(C3H) in both LTRs. In hopes of boosting expression upon transfection of the construct into cells, we placed the transcriptional enhancer element from Mo-MLV ≈0.3 kb upstream of the 5′ LTR. Similar positioning of a murine sarcoma virus enhancer with an MMTV LTR linked to a reporter gene resulted in ≈25-fold higher expression than without the enhancer in the presence of dexamethasone (14).

From this MMTV construct we have derived DNA to produce a replication-defective MMTV-neo vector by replacing *pol* and *env* with a portion of pZIPneoSV(X), a Mo-MLV-based shuttle vector (6) (Fig. 1). This portion contains the splice acceptor from Mo-MLV *env*, the Tn*5* *neo* gene, and the origins of replication from SV40 and pBR322. Thus, the MMTV-neo vector DNA has the potential to confer resistance to the antibiotics G418 (in mammalian cells) and kanamycin (in bacteria) and to be amplified in the presence of tumor antigen and efficiently recloned in *Escherichia coli*, as can pZIPneoSV(X) (6). This vector contains a single

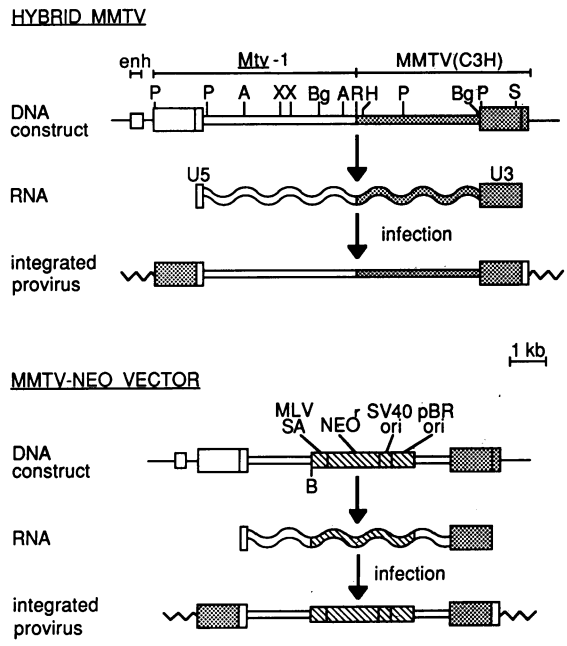

FIG. 1. Schematic representation of the hybrid MMTV and derivative MMTV-neo vector proviruses. The hybrid MMTV provirus consists of *Mtv-1* DNA 5′ of the unique *Eco*RI (R) site in the viral *pol* gene and DNA from the MMTV(C3H) provirus 3′ of this site (shaded regions). The transcriptional enhancer element (enh) from Mo-MLV (a 225-base-pair *Sau*IIIA–*Xba* I fragment) was placed just upstream of the 5′ LTR to boost initial expression. Upon infection of cells with the resulting virus, MMTV(C3H)-specific transcriptional control information in *U3* from the 3′ LTR will be present in the newly synthesized 5′ LTR. The MMTV-neo vector is a derivative of the hybrid MMTV provirus in which the *pol* and *env* genes have been replaced by sequences from pZIPneoSV(X) (hatched region) containing the Mo-MLV splice acceptor (MLV SA), the *neo* gene (NEO^r), and the origins of replication from SV40 and pBR322 (SV40 ori and pBR ori). Restriction sites used in this study are: A, *Acc* I; B, *Bam*HI; Bg, *Bgl* II; R, *Eco*RI; H, *Hin*dIII; P, *Pst* I; S, *Sac* I; X, *Xho* I.

*Bam*HI site immediately 5′ of the Mo-MLV splice acceptor for insertion of additional foreign genes.

MMTV-neo Vector Is Infectious with MMTV(C3H) Helper. We first tested our MMTV-neo vector by transfecting the DNA construct into D108, an XC cell line already producing MMTV(C3H) as a result of prior virus infection (9). Nine G418-resistant colonies were isolated after transfection and screened for MMTV-neo vector virus production by hybridization to RNA from pelleted virus particles with a *neo* hybridization probe. A colony that scored positive in this assay was selected (D108-9) and tested for transmissability of neomycin resistance.

To test for infectious MMTV-neo virus, G418-resistant donor cells, such as D108-9, were cocultivated with hygromycin B-resistant XC recipient cells, followed by selection with both drugs for infected recipient cells. Cocultivation was used instead of infection with a cell-free virus stock because of the generally poor specific infectivity of MMTV *in vitro* and the increased efficiency of infection by cocultivation. However, a caveat to cocultivation is the possibility of chromosome transfer by cell fusion. For this reason we have shown that all apparent infections were genuine by demonstration of new proviruses.

Synthesis of new hybrid MMTV proviruses by reverse transcription can be demonstrated by digestion of infected cell DNA with *Sac* I. The *Sac* I site present only in *U3* of the 3′ LTR of the DNA construct is present in both LTRs of newly synthesized proviruses (Fig. 2 *Lower*). Analysis of *Sac* I-digested genomic DNA hybridized with a *neo* probe should then reveal a 7.0-kb fragment corresponding to a unit-length

provirus in all infected cells; *Sac* I-digested donor cell DNA should show larger fragments hybridizing to the *neo* probe, since no *Sac* I site is present in the 5′ LTR of the transfected DNA. Digestion of DNA from donor and infected cells gave the expected results: unit length (7.0 kb) *neo* fragments from infected-cell DNA, providing evidence of infection, and larger fragments from the donor cell DNA (Fig. 2). *Hin*dIII digests of these DNAs tested with the *neo* probe (Fig. 2) reveal the independent origins of each infected clone and the donor cells. No fragments were detected in DNA from the uninfected recipient cells. Thus, the MMTV-neo vector has successfully infected XC cells when complemented by wild-type MMTV.

MMTV-neo Vector Is Infectious when Using the Hybrid MMTV DNA as Helper. To test the ability of our hybrid MMTV DNA to provide helper functions for the MMTV-neo vector, we created cell lines expressing both hybrid and vector constructs by cotransfecting them into XC cells and selecting with G418. A cell line producing vector virions was chosen by hybridization of pelleted virion RNA with a *neo* probe for further use as a donor cell line. These cells were cocultivated with hygromycin B-resistant XC recipient cells, and G418- and hygromycin B-resistant cells were selected and tested for new proviruses indicative of virus infection. Again a 7.0-kb *Sac* I fragment was detected with the *neo* probe in digests of DNA from selected cells, demonstrating that the hybrid MMTV is capable of providing all products necessary for production of infectious virus vector (Fig. 3). The provirus in the larger *Sac* I fragment from clone 8 may reflect a proviral mutation but was not further investigated. As expected, the *Sac* I digest of DNA from donor cells contained *neo* fragments larger than 7.0 kb.

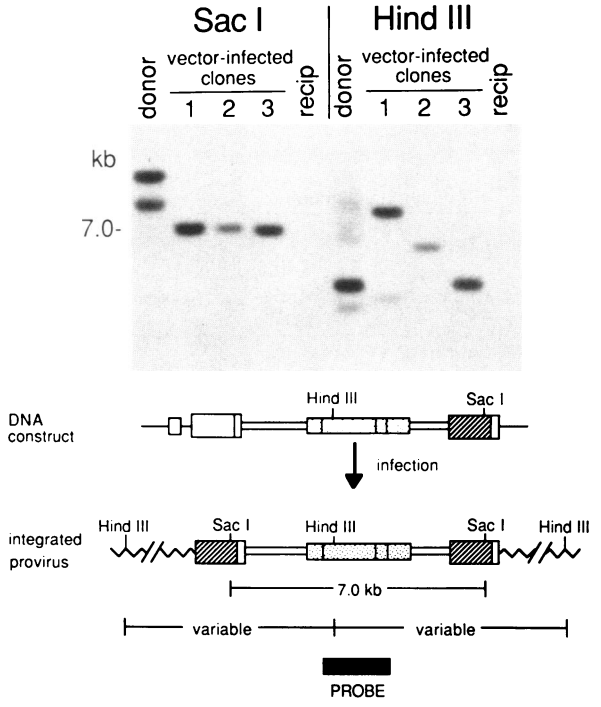

FIG. 2. Detection of MMTV-neo vector proviruses in vector-infected cells with MMTV(C3H) as helper virus. (*Upper*) MMTV(C3H)-infected XC cells, transfected with the MMTV-neo vector construct, were cocultivated with hygromycin B-resistant XC cells. High molecular weight DNAs were isolated from XC cells resistant to both antibiotics, digested with *Sac* I or *Hin*dIII, separated by gel electrophoresis, blotted to a nylon membrane, and hybridized to a vector-specific *neo* probe; 7.0 kb indicates unit-length *Sac* I-digested *neo* fragments. recip, Recipient. (*Lower*) The diagram identifies the restriction site locations in the vector construct and in an integrated vector provirus postinfection and shows the origins of the fragments. Note that *U3* sequences containing the *Sac* I site from the 3′ LTR of the DNA construct (hatched) are copied to the 5′ LTR of the integrated provirus during infection.

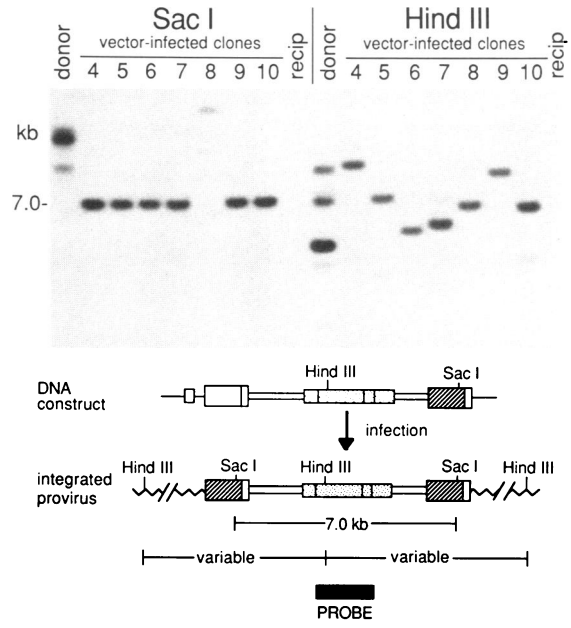

FIG. 3. Detection of MMTV-neo vector proviruses in vector-infected cells with the hybrid MMTV as helper virus. Analysis (*Upper*) and diagram (*Lower*) are as outlined in the legend to Fig. 2 except that the donor cells were XC cells transfected with constructs of both MMTV-neo vector and hybrid MMTV proviruses. recip, Recipient.

The variably sized *Hin*dIII fragments that hybridize with *neo* probe document that the selected cell lines were independently infected (Fig. 3).

Hybrid MMTV Is Infectious. We wished to determine if the hybrid MMTV construct was itself capable of producing infectious virus. Because the hybrid construct can provide helper functions for the MMTV-neo vector and because hybrid MMTV virions are present in the medium of cell lines producing vector virus (data not shown), we asked whether some of the vector-infected cells were also infected by the hybrid MMTV helper virus. The filters used for hybridization in Fig. 3 were stripped of probe and incubated with a probe for the MMTV *env* region (which does not hybridize to vector sequences). Several of the MMTV-neo vector-infected clones also contained at least one newly synthesized hybrid MMTV provirus, as evidenced by an 8.5-kb *Sac* I fragment (Fig. 4). This finding and analysis of *Hin*dIII digests with the *env* probe showing that each cell contains one to three uniquely located provirus(es) (Fig. 4) indicate that the hybrid MMTV is infectious.

Hybrid MMTV and MMTV-neo Vector RNAs are Dexamethasone-Inducible. One of the potential advantages of an MMTV-based vector system is the option of regulating transcription with glucocorticoid hormones (15). To test this aspect of our MMTV proviruses, we examined MMTV RNA in cell lines expressing the hybrid MMTV, the MMTV-neo vector, or both proviruses (Fig. 5, cell lines C, B, and A, respectively) after growth in the presence or absence of dexamethasone. Cells grown continually in medium containing 0.1 μM dexamethasone (Fig. 5, lanes 3, 6, and 8) had levels of hybrid and vector virus RNAs 10- to 100-fold higher than concentrations of viral RNAs in the same cell lines grown without dexamethasone for 7 days (Fig. 5, lanes 1, 4, and 7). Also, growth of cells in the absence of dexamethasone for 7 days followed by 2 days in the presence of the hormone resulted in increased amounts of all viral RNAs (Fig. 5, lanes 2 and 5), indicating that expression remains inducible after a period of deinduction. These induced levels are similar to those observed in cells grown in the constant presence of dexamethasone. These results show that elements responsi-

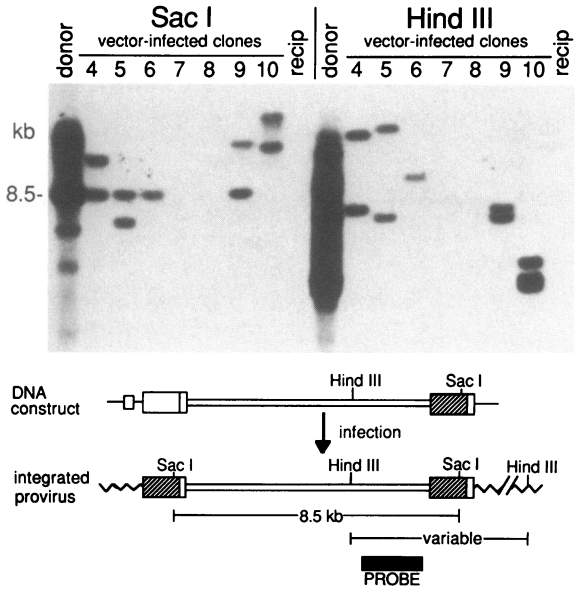

FIG. 4. Detection of hybrid MMTV proviruses in MMTV-neo vector-infected cells. (*Upper*) The DNA blot from Fig. 3 was stripped of probe and hybridized with a probe for the *env* region of the hybrid MMTV. The 8.5-kb *Sac* I fragment is evidence of a newly synthesized hybrid MMTV provirus. recip, Recipient. (*Lower*) The diagram shows the origins of fragments as described in Fig. 2.

ble for glucocorticoid induction in newly integrated hybrid and vector proviruses are functional.

Hybrid MMTV Is Tumorigenic. As a further test of the biological activity of the hybrid MMTV, we infected 3- to 4-week-old female BALB/cJ mice by injection of XC cell lines producing the hybrid MMTV. The recipient mice were forcebred and observed for tumor formation. Eight of nine infected mice developed mammary tumors within 1 year; the earliest tumors arose during the third pregnancy, 3–4 months postinfection, in 3 mice. The average tumor latency in these eight mice was 6.5 months. Although most tumors were harvested upon detection, several small tumors not removed immediately were observed to be pregnancy-dependent for growth and receded between pregnancies. Six control mice, which received XC cells not producing virus, did not develop tumors.

Seven tumors were collected from six mice, and the DNA was analyzed to ascertain whether the tumor originated from

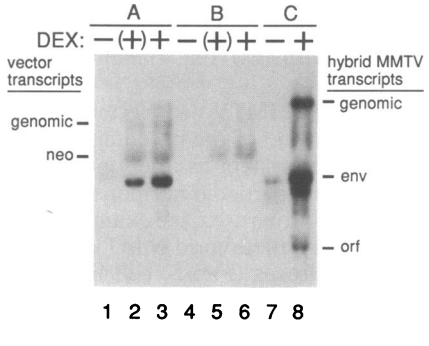

FIG. 5. Dexamethasone inducibility of hybrid MMTV and MMTV-neo vector RNAs. Total cellular RNAs (25 μg) extracted from three infected cell lines (A, B, and C) grown in the presence or absence of 0.1 μM dexamethasone were separated on an agarose gel, blotted to a nylon membrane, and hybridized with a probe for MMTV *env* and LTR sequences. RNAs in lanes: 1, 4, and 7, from cells grown without dexamethasone for 7 days; 2 and 5, from cells treated as in lanes 1, 4, and 7 but refed with medium containing dexamethasone for 2 days; 3, 6, and 8, from cells grown constantly in the presence of dexamethasone. The ethidium bromide-stained gel showed that all lanes contained equal amounts of RNA (data not shown). orf, open reading frame of 3′ LTR.

mouse cells infected with hybrid MMTV. We chose a probe and restriction enzymes that would allow distinction of our hybrid MMTV from the three endogenous MMTV proviruses present in BALB/c mice (*Mtv-6, -8,* and *-9*) and from exogenous MMTV(C3H) virus (Fig. 6). After digestion with *Pst* I and *Bgl* II, endogenous *Mtv-8* and *Mtv-9* proviruses yielded 3.0-, 2.7-, and 2.1-kb fragments detected with a probe from the *gag-pol* region (Fig. 6, lane 1); *Mtv-6* did not hybridize with this probe. Any unexpected proviruses from exogenous MMTV(C3H) would produce *gag-pol* fragments of 2.3 and 1.5 kb (Fig. 6, lane 10). However, the hybrid MMTV DNA was represented by 3.0- and 2.3-kb fragments (Fig. 6, lane 9). Each of the tumors exhibited evidence of an acquired hybrid MMTV provirus(es), but not exogenous MMTV(C3H) proviruses, by the presence of a unique 2.3-kb fragment, an increased abundance of 3.0-kb fragments, and absence of 1.5-kb fragments (lanes 2–8; see diagram).

To establish the independent origin and number of new proviruses present in each tumor, we examined the tumor DNAs with *Bgl* II and a viral probe able to recognize host–virus junction fragments (Fig. 7). In addition to fragments of endogenous proviruses (Fig. 7 *Upper*, lane 1) and internal 4.1-kb fragments of acquired proviruses (Fig. 7, lanes 2–8), we detected additional fragments of expected size (≥4.4 kb; Fig. 7 *Upper*, lanes 2–8, and *Lower*) corresponding to individual proviral junction fragments in each of the tumors.

The identification of hybrid MMTV proviruses clonally integrated in mammary tumors of infected mice strongly implicates the hybrid MMTV provirus in the genesis of these tumors. The presence of BALB/c endogenous proviruses in the tumor DNAs (Figs. 6 and 7) confirms that the tumors are of mouse origin and did not arise from the injected rat XC cells. Moreover, ethidium bromide staining of restriction

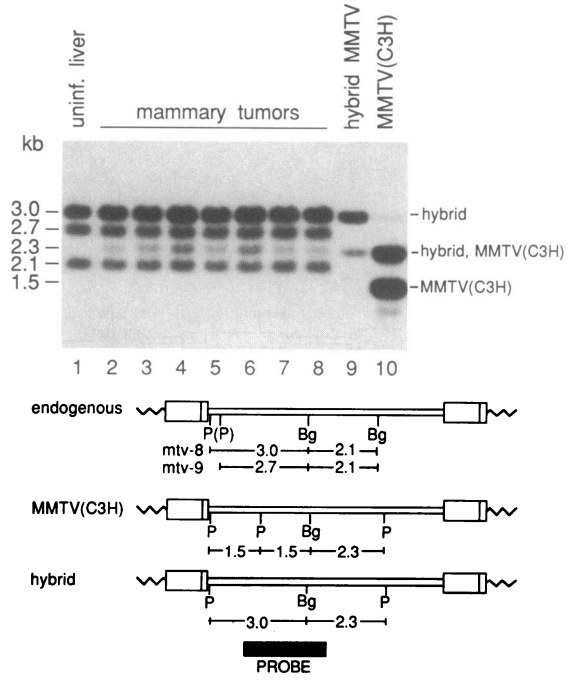

FIG. 6. Detection of hybrid MMTV proviruses in mammary tumors of infected BALB/cJ mice. (*Upper*) High molecular weight DNAs from normal BALB/cJ liver (lane 1), seven mammary tumors (lanes 2–8), and rat XC cells infected with the hybrid MMTV (lane 9) or MMTV(C3H) (lane 10) were digested with *Pst* I and *Bgl* II and subjected to DNA blot analysis using the hybridization probe depicted in the diagram. uninf., Uninfected. (*Lower*) Origins of the fragments are shown in the diagram. Only pertinent *Pst* I (P) and *Bgl* II (Bg) sites are shown. The presence of the 2.3-kb fragment and the absence of a 1.5-kb fragment are indicative of the hybrid MMTV provirus in tumors.

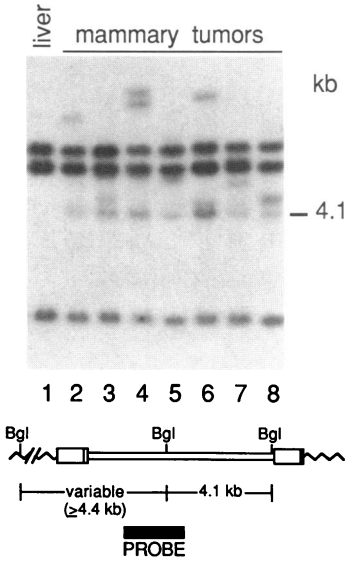

FIG. 7. Demonstration that tumors are clonal with respect to hybrid MMTV integrations. (*Upper*) Normal liver (lane 1) and mammary tumor (lanes 2–8) DNAs were digested with *Bgl* II and analyzed as in Fig. 6. (*Lower*) The probe detects a 4.1-kb internal fragment from hybrid MMTV proviruses and the 5′ host–virus junction fragment (≥4.4 kb) for each acquired provirus.

endonuclease-digested tumor DNAs in the above analyses and others showed repeat patterns indicative of mouse DNA (data not shown).

DISCUSSION

To circumvent the difficulty in cloning the *gag* region from milk-borne MMTVs, we have constructed a hybrid provirus utilizing the clonable *gag* and adjacent regions from provirus *Mtv-1* together with sequences from an MMTV(C3H) provirus. We have shown that this provirus produces infectious and tumorigenic virus particles when introduced into rat cells by transfection. We also derived a replication-defective MMTV-neo vector provirus from the hybrid MMTV and produced vector virus capable of transferring G418 resistance via infection when supplied with viral proteins from either the hybrid MMTV or MMTV(C3H). Both hybrid and vector MMTV proviruses are transcriptionally inducible by dexamethasone in infected cells.

Mice infected with our hybrid MMTV efficiently develop mammary tumors with an average latency (6.5 months) similar to that observed in MMTV(C3H)-infected BALB/c mice (7–8 months; ref. 16). Thus, the hybrid provirus produces virus that apparently retains the normal pathogenic properties of the parental MMTV(C3H) from which most of its genome is derived. The method of infection used here—direct injection of heterologous cells producing hybrid MMTV—has the advantage of simplicity (by avoiding virus concentration steps and the concomitant loss of activity), while apparently not compromising infectivity. This method may be of use in other viral systems in which the intended host is immunologically competent to reject input producer cells.

Although several groups have cloned full-length endogenous or exogenous proviruses without obvious rearrangements (17–20), these have not been shown to produce infectious or tumorigenic virions. Recently, a pathogenic clone of an exogenous provirus from a GR mouse was isolated in λ phage, but the *gag* region of this provirus remains resistant to cloning in plasmids (D. W. Morris of the University of California, Davis, personal communication). Gunzberg and Salmons and co-workers have used a strategy similar to that presented here for construction of a clonable MMTV provirus and vector (21, 22); however, the infectivity and pathogenicity of particles produced by that chimeric provirus were not described.

The fully active MMTV and vector system described here will allow several important questions to be addressed. For instance, the role of the conserved open reading frame of the LTR (23, 24) and the role of other sequences in viral replication and tumorigenesis may now be examined directly by site-directed mutagenesis. The MMTV-neo vector has obvious potential utility as a hormonally regulated retrovirus vector for discerning the effects of conditional expression of genes transferred into cultured cells. It could also be used to direct genes of interest to mouse tissues known to be susceptible to MMTV infection: mammary glands and lymphoid tissues (25, 26).

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